PV tree was developed where pressure was modeled as the product of chamber elastance and volume. An analytical solution of the unsteady Bernoulli equation was achieved by linearizing the equation. The solution equation consisted of a cosine function, which captures *vis a tergo*, and a sine function, which captures *vis a fronte*. Time-varying systolic PV velocity, ut(t) – cms, is a function of the following variables: total atrial suction created by atrial relaxation (ΔP_{ar} – mmHg) and mitral annular descent (ΔP_{md} – mmHg), atrial (E_a – mmHg/cc) and PV elastance (E_{pv}), net atrial – PV elastance ($E_{net} = E_a + E_{pv}$), the average rate of PV tree replenishment from the capillaries (Q_L – cm^3/s), total PV cross-sectional area at the atrial junction (A – cm^2), and PV inertance (pL_{eff} – g/cm^2). This model found that the peak velocity and integral of systolic PV flow increased as atrial suction and PV tree replenishment increased, but decreased as atrial and PV elastance, PV inertance, and PV cross-sectional area increased. Also, this model predicts aspects of systolic PV flow that are consistent with experimental and clinical observations: (i) the *vis a tergo* component of systolic PV flow is minimized by compliant PVs (small PV elastance); (ii) atrial events, so-called *vis a fronte*, dominate the magnitude of systolic PV flow; (iii) systolic PV flow is reduced by elevations in atrial pressure but *only* when compliance is also reduced; (iv) compensatory increases in atrial suction and PV tree replenishment may overcome reductions in systolic PV flow due to elevated atrial pressures; (v) PV inertance and area are important determinants of the magnitude and timing of PV flow.

$$U(t) = \frac{Q_L E_{pv}}{E_{net} A}\left[1 - \cos\sqrt{\frac{E_{net} A}{\rho L_{eff}}}(t)\right] + \frac{\Delta P_{ar} + \Delta P_{md}}{\sqrt{\rho L_{eff} E_{net} A}} \sin\sqrt{\frac{E_{net} A}{\rho L_{eff}}}(t)$$

11:15

407-4 Differential Sympathetic Neural Control of Muscle Oxygenation in Resting and Exercising Human Skeletal Muscle

Jim Hansen, Gail D. Thomas, William J. Parsons, Ronald G. Victor. *UT Southwestern, Dallas, Texas*

Although the muscle metaboreflex triggers increases in muscle sympathetic nerve activity (SNA) targeted to both resting and exercising human skeletal muscle, our recent rat studies advance the hypothesis that the vasoconstriction normally evoked by increased muscle SNA is selectively attenuated in the contracting muscle, thereby optimizing muscle perfusion. The ability to continuously measure muscle oxygenation (cytochrome a,a_3 redox state and tissue oxygen stores ($t(HbO_2 + MbO_2)$) in exercising muscle with near infrared spectroscopy while simultaneously measuring muscle SNA (microneurography) provided a new opportunity to test this hypothesis in humans. Specifically, we asked if a reflex increase in muscle SNA decreases muscle oxygenation in resting, but not in exercising muscle. In 10 healthy humans, we measured oxygenation in forearm muscle during reflex increases in muscle SNA evoked by non-hypotensive lower body negative pressure (LBNP, –20 mmHg) at rest and during mild rhythmic handgrip (5 min 20% of maximum). At rest, LBNP increased muscle SNA by 292 ± 72% (mean ± SE) and decreased $t(HbO_2 + MbO_2)$ by 10 ± 1% ($p < 0.05$) of the maximal decrease (assessed with complete forearm ischemia). This decrease in $t(HbO_2 + MbO_2)$ in the resting forearm was sympathetically-mediated because it was abolished by local sympathetic block with bretylium. Handgrip alone caused no change in muscle SNA, but rapidly decreased $t(HbO_2 + MbO_2)$ to a steady state 28 ± 4% below baseline. Importantly, when superimposed on handgrip, LBNP increased MSNA by 305 ± 59%, but now had no effect on tissue oxygenation in the exercising muscle ($\Delta t(HbO_2 + MbO_2)$ = +0.4 ± 3%, p = ns). These data provide direct evidence in humans that reflex sympathetic activation decreases oxygenation in resting, but not in exercising human skeletal muscle. This mechanism would optimize oxygenation of active muscle when the muscle metaboreflex is engaged.

11:30

407-5 The Pharmaco-Genetics of Congenital Cardiac Defects in Retinoic Acid Receptor Deficient Mouse Embryos

Henry M. Sucov, Vicky LaMorte, Jiangming Luo, Vincent Giguere, Ronald M. Evans. *Univ. of Southern California School of Medicine, Los Angeles, CA*

Germline mutations in several of the genes which encode receptors for the signaling molecule retinoic acid (RA) have been previously established in mice. Cardiac phenotypes which emerge in mutant embryos include defects in the ventricular chamber, conus, truncus, and aortic arch arteries. Based on new experimental and theoretical observations, the following conclusions have been drawn concerning the pharmacology of RA receptor function in mediating complex aspects of cardiac morphogenesis: (1) the level of functional receptor, known to be a heterodimer of RAR and RXR monomers, is dictated by equilibrium considerations; (2) in the tissue involved in septation of the truncus, the levels of RAR and RXR monomers are roughly comparable, explaining the emergence of persistent truncus arteriosus only after combined mutation of RAR and RXR; (3) target genes of RA action critical in the morphogenesis of the ventricular chamber contain receptor binding sites in their promoters of moderate, rather than high, affinity; this is also likely to be true in most other cases of RA action; (4) differentiation of the conus cushions is a significantly different response to RA than is morphogenesis of either the truncus or the ventricular chamber, in that the conus is sensitive to both insufficient or excess RA, whereas the truncus and ventricular chamber are sensitive to only RA deficiency; (5) the pharmacology of the RA receptors may in some cases make it possible to reverse the consequences of receptor mutation by treatment with exogenous RA; this approach was not successful in rescuing the hypoplastic ventricular phenotype, indicating that receptor mutation also supresses the inducible RA response to below the required threshold in this tissue. These conclusions make testable predictions concerning the molecular mechanisms of normal and pathological cardiac morphogenesis, which will be evaluated through future molecular analysis.

Discussion 11:45

408 Young Investigators Awards Competition – Molecular and Cellular Cardiology

Monday, March 25, 1996, 2:00 p.m.–3:30 p.m.
Orange County Convention Center, Room 231

2:00

408-1 Localization of the Gene for Familial Idiopathic Dilated Cardiomyopathy to Chromosome 1q32

Jean-Bernard Durand, Linda L. Bachinski, Lisa Beiling, Grazyna Z. Czernuszewicz, Antoine B. Abchee, Qun Tao Yu, Rita Hill, Jonah Ifegwu, A.J. Marian, Ramon Brugada, Steven Daiger, Jane M. Gregoritch, Jeff Anderson, Miguel Quiñones, Jeffrey A. Towbin, Robert Roberts. *Baylor College of Medicine, Houston, Texas*

Cardiac failure is the fastest growing cardiovascular condition in the Western world with over 400,000 new cases per year in the U.S. The cost in the U.S. for cardiac transplantation alone is over $200 million and for all cardiac failure over $10 billion. Dilated cardiomyopathy (DCM), the most common cause of heart failure, is a primary cardiac disease characterized by ventricular dilatation and impaired systolic contraction and manifested clinically by either sudden death or pump failure. The cause for DCM is unknown (idiopathic, IDCM) with 20% estimated to be inherited. To identify the genetic defect for IDCM one must first map the chromosomal locus. Thus, we studied a four generation kindred with ten members having IDCM, detected by echocardiography. IDCM segregated as a highly penetrant autosomal dominant disorder. A genome search was performed using short tandem repeat polymorphic (STRP) markers. Two-point and multi-point linkage analysis was conducted *assuming penetrance to be 90%*. Approximately 90% of the genome was excluded by multi-point analysis of > 400 markers. Peak lod score of 5.78 was obtained at the marker DIS414 with θ = 0% recombination. Peak multi-point *lod score, also at D1S414*, was 6.37. Haplotype analysis identified recombination events between the disease gene and polymorphic markers in seven individuals. The region of 1q common to all ten affected individuals is flanked by *markers D1S249 and D1S549* which are approximately 20 cM apart. Reasonable candidates including myosin-binding protein-H, MEF-2D, renin and plasma membrane calcium transporting ATPase, are known to be localized to this region and studies are now underway using the positional candidate approach to isolate and identify the responsible gene. Identification of the genetic defect should elucidate the molecular basis for familial DCM, providing a means for precise diagnosis and screening of asymptomatic individuals at risk prior to the development of this disease, which is a necessary step in our ultimate goal of providing effective prevention and treatment of this disease.

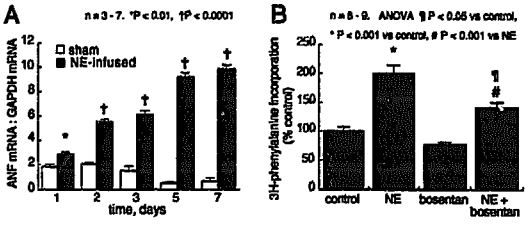
2:15

408-2 A Functional Role for Endothelin-1 in Norepinephrine-Induced Ventricular Hypertrophy *in Vivo* and *in Vitro*

Samer Kaddoura. *MRCP, National Heart & Lung Institute, London, United Kingdom*

Background Endothelin-1 (ET-1) has potent effects upon cell growth. We tested the hypothesis that endogenous ET-1 plays a functional role in norepinephrine (NE)-induced ventricular hypertrophy by studying physical indices

and molecular markers of hypertrophy, ventricular and non-cardiac expression of ET-1 mRNA and the effects of bosentan, an orally-active competitive ET_A and ET_B receptor antagonist without adrenoceptor antagonism. *Methods and Results* Initially, a rat model of ventricular hypertrophy due to continuous infusion of NE (600 μg/kg/h up to 7 days) by subcutaneous osmotic pumps was used. Male Sprague-Dawley rats (175–200 g, n = 70) were divided into 4 groups: 1. Sham-operated, 2. NE-infused, 3. Sham-operated given bosentan (100 mg/kg/day by gavage), 4. NE-infused given bosentan. NE caused a 35-fold increase in ventricular ET-1 mRNA within 1 day (sham – ET-1:GAPDH mRNA, 0.01 ± 0.01 at 1 day, n = 6 vs NE – ET-1:GAPDH mRNA, 0.35 ± 0.08, n = 8, P < 0.01), an effect not seen in lung, kidney or skeletal muscle. NE also caused significant increases in ventricular weight, RNA:protein, and expression of mRNAs for atrial natriuretic factor (ANF), beta-myosin heavy chain and skeletal alpha-actin, which in adult ventricle are indicators of hypertrophy (fig. A). Bosentan blocked NE-induced hypertrophy at 5 days. *In vitro*, NE increased expression of ET-1 mRNA by cultured ventricular myocytes and led to hypertrophy with increased cell size, sarcomerization and expression of ANF mRNA. Myocyte protein content and [3H]-phenylalanine incorporation also increased, effects blocked by 10 μM bosentan (fig. B). *Conclusion* ET-1 plays a direct role in mediating NE-induced hypertrophy with 'cross-talk' between these systems *in vivo* and *in vitro*.



2:30

408-3 Cis/Trans Regulation of Vascular Endothelial Growth Factor mRNA Stability by Hypoxia

Andrew P. Levy, Nina S. Levy, Mark A. Goldberg. *Boston University School of Medicine, Boston, MA*

Hypoxia has been shown to be an important stimulus for the formation of new blood vessels in the coronary collateral circulation in coronary artery disease. Vascular endothelial growth factor, a potent angiogenic factor, is regulated by hypoxia in vitro and in vivo. The major control point for the hypoxic induction of the VEGF gene is the regulation of the steady-state level of the mRNA which is determined by the relative rates of mRNA synthesis and decay. We previously demonstrated a discrepancy between the transcription rate and the steady-state mRNA level induced by hypoxia. This lead us to examine the post-transcriptional regulation of VEGF expression. Actinomycin D experiments revealed that hypoxia increased VEGF mRNA half-life from 43 ± 6 minutes to 106 ± 9 minutes. Using an in vitro mRNA degradation assay the half-life of VEGF mRNA 3′ untranslated region (UTR) transcripts were also found to be increased when incubated with hypoxic versus normoxic extracts. Both cis-regulatory elements involved in VEGF mRNA degradation under normoxic conditions and in increased stabilization under hypoxic conditions were mapped using this degradation assay. Hypoxia-induced proteins were identified by RNA electromobility shift assay that bound to the sequences in the VEGF 3′ UTR which mediated increased stability in the degradation assay. The binding site of one of these proteins was localized in the VEGF 3′ UTR within 20 bases of the nonameric sequence, UUAUUUAUU, previously shown to mediate the rapid degradation of multiple cytokine and oncogene mRNAs. This suggests a novel mechanism whereby these hypoxia-inducible proteins stabilize VEGF mRNA by interfering with the nonameric site binding to its cognate binding protein(s). Furthermore, genistein, a tyrosine kinase inhibitor which was recently shown to inhibit the hypoxic induction of VEGF mRNA through its action on src, blocked the hypoxia-induced stabilization of VEGF 3′ UTR transcripts and inhibited hypoxia-induced protein binding to the VEGF 3′ UTR while having no effect on the hypoxia-induced increase in transcription of the VEGF gene. This suggests a signal cascade through src/raf and MAP kinase that has as its terminal event a protein that binds to VEGF mRNA and mediates its hypoxic stabilization. An understanding of the molecular basis of the regulation of VEGF by hypoxia forms the essential groundwork for the rational design of pharmacological agents to increase VEGF expression and thereby augment neovascularization in regions of ischemic myocardium.

2:45

408-4 A Novel Atherogenic Activity of Lipoprotein(a): Induction of Monocyte Chemotactic Activity in Human Umbilical Vein Endothelial Cells

Michael Poon, Xiaoxia Zhang, Mark B. Taubman, Peter C. Harpel. *Mount Sinai School of Medicine, New York, NY*

Elevated levels of lipoprotein(a) (Lp(a)) are associated with increased risk of cardiovascular mortality and morbidity. Recruitment of monocytes to the blood vessel wall is an early event in the pathogenesis of atherosclerosis. We now report that Lp(a) induces the secretion of monocyte chemotactic activity (MCA) by human umbilical vein endothelial cells (HUVEC). MCA was assayed using a modified Boyden Chamber. Minimal MCA was produced by HUVEC incubated in serum-free medium. Lp(a) induced MCA in HUVEC beginning at 30 min and peaking at 2–3 hr. Peak levels were ≈80% of those seen with f-met-leu-phe, a potent monocyte chemoattractant. The induction of MCA was concentration dependent, with maximum MCA seen at 100 μg/ml of Lp(a). LDL from the same donor failed to induce MCA. In addition, Lp(a) had no direct chemotactic activity for monocytes. Polymyxin B failed to inhibit the stimulatory effect of Lp(a), suggesting that lipopolysaccharide was not the active agent. Checkerboard analysis documented that the activity presents in Lp(a)-conditioned medium was primarily chemotactic rather than chemokinetic. Concomitant treatment of HUVEC with Lp(a) and either 10 μM actinomycin D or cycloheximide completely inhibited the secretion of MCA into the culture medium, indicating that protein and RNA synthesis were required. It has been previously shown that oxidized LDL induces monocyte chemotactic protein-1 (MCP-1) mRNA and activity in human aortic endothelial cells. In the current study, Northern blot analysis showed that Lp(a) did not induce MCP-1 mRNA in HUVEC, indicating that Lp(a) may induce a chemoattractant other than MCP-1. These studies suggest that Lp(a) may be involved in the recruitment of monocytes to the vessel wall and provide a novel mechanism for the participation of Lp(a) in the atherogenic process.

3:00

408-5 Genetic Expression of Endothelial and Inducible Forms of Nitric Oxide Synthase in the Normal and Failing Human Hearts

Chi-Ming Wei, Shi-Wen Jiang, Richard C. Daly, Christopher G. A. McGregor. *Mayo Clinic, Rochester, MN*

Nitric oxide (NO) is a potent endothelium-derived relaxing factor which also may modulate cardiomyocyte inotropism and growth via increasing the cGMP level. While both the endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) isoforms have been detected in non-human mammalian tissues, expression and localization of the eNOS and iNOS in the normal and failing human heart are poorly defined. The present study was designed to investigate the eNOS and iNOS in human cardiac tissues in the presence and absence of congestive heart failure (CHF). Normal and failing atrial tissue were obtained from twelve cardiac donors and twelve end-stage heart failure patients undergoing cardiac transplantation. eNOS and iNOS expression and localization were investigated utilizing Northern blot analysis, in situ hybridization and immunohistochemistry. Northern blot analysis and in situ hybridization demonstrated similar levels of the eNOS and iNOS mRNA are present in cardiomyocytes from normal and failing hearts. Positive immunohistochemical staining was observed within the cytoplasm of cardiomyocytes. No significant different eNOS and iNOS immunoreactivities between normal and failing human myocardium were detected. The present studies demonstrated for the first time the genetic expression and distribution of eNOS and iNOS in the normal and failing human hearts. These studies suggest that the NOS mediated paracrine and autocrine pathway may continue to control the myocardial function in the failing human hearts.

Discussion 3:15